Reviewer's report

Title: Isolation of non-tuberculous mycobacteria (NTM) from pastoral ecosystems of Uganda: Public Health significance

Version: 1 Date: 29 December 2010

Reviewer: Rachel Thomson

Reviewer's report:

I congratulate the authors on a difficult study performed in a developing country. The findings are relevant to the field of NTM in the environment and have human and veterinary interest.

There are however a number of ways this manuscript can be improved.

Major Compulsory revisions.

Introduction.

I found the introduction to be long and not particularly focused to the research question. There could be less generalized comments about NTM disease and more discussion about which NTM have been found to cause disease in humans in Uganda, and how those cases presented and were diagnosed. The aim of the study is a little vague and could be elaborated. Some discussion about the methods of isolation of mycobacteria is also needed, to support the methods chosen by the authors. The case definition is poor, and whilst I realize the microbiological confirmation of disease is not always provided in developing countries, some criteria for selection of cases is needed.

Methods.

The methods are well described, though some of the information provided is superfluous to the discussion of results. E.g. the lat/longitudes and populations of the districts are not particularly relevant unless they are useful in the analysis - so cases per 100 000 population could be provided, and an analysis of yield of mycobacteria against geographical variables etc

Sample size determination of the questionnaire survey: need to separate NTM and TB incidence/prevalence. As the numbers are likely to be contaminated by cases of M. tuberculosis, you need to be more clear about the case definition.

How did you identify a household with a case – and how was that case diagnosed? What form of disease did they have?

It took me a while to work out that the survey results were being compared to the isolation of NTM from the environment – this could be more clearly articulated in the aims.

Questionnaire: How was the questionnaire “standardized”?

You state “alongside the questionnaire, samples from water…” – were the
samples collected the same day as the questionnaire were administered or separate – what was the time frame? How did the timing of administration of questionnaire and the sample collection relate to the timing of the diagnosis/illness of the index case in the household?

Samples: what was the rational behind the chosen methods of sample size (30ml), decontamination, concentration and culture and how might these methods have impacted on your yield?

Surface layer soil was not collected – how deep did you go to collect the soil samples then, and how close to the water edge were they?

It wasn’t clear from reading how the water samples were represented until I read the supplements at the end. You could include in the text (and refer to the table) to better describe the number of household vs dam vs stream samples. There were 231 households, yet only 130 drinking water samples collected? why

Given the finding of the seasonal differences in yield, I think you need to show when the samples were collected – or in the results, show denominators/percent rather than absolute numbers per month.

Isolation and identification: why was NCBI Blast chosen over other databases such as Genbank and RIDOM? What did you do with isolates that came back as M. species? what level of match did you accept?

RESULTS.
Microbiological results are not well expressed. What proportion of samples had positive growth, (and subsequent ZN positivity), no growth or contamination/overgrowth by non acid fast organisms?

As mentioned the yield for each month needs to be expressed rather than absolute numbers. You could examine the yield per month from each type of sample also.

Because of the low yield of mycobacteria and the bias introduced by the seasonal variation in yield, I’m not sure that the findings from the multivariable logistic regression proportion are valid. The wording “drinking of untreated water relative to treated” is misleading, as it implies ‘drinking’ the water is a risk factor for disease acquisition, when I think you are meaning that the ‘untreated water was more likely to contain mycobacteria that treated”. The use of the phrase “risk factors for exposure” is also confusing. You can really only say “risk factors for presence of mycobacteria” as exposure requires human activity which you did not assess in detail.

DISCUSSION.
Need to address the results in more detail – ie factors that may have affected the yield – methods chosen, seasonal sampling, etc

And how these may have affected what you can conclude

“An average of 15% of the environmental samples contained mycobacteria” – average of what? reword
Yield from soils around water sources highest in this study – could this be because the decontamination method is best suited for soils, and perhaps be too strong for drinking water?

The third paragraph is one of the major limitations to this study and needs to be stated as such.

Rather that focus on an exhaustive description of how the species you have found have also been found in humans, you would be better to discuss your own results and the implications they have for the people studied and whether they are generalisable to other populations.

The last three paragraphs of discussion need attention. The term “exposure risk factor” is misleading and the first sentence is therefore confusing. All you can conclude is that these are factors that may be associated with the environmental isolation of mycobacteria. You state that “Other studies show that contaminated water sources are known to provide the foci of mycobacterial infections in humans and animals” Be careful here. Contaminated bronchoscope cleaning fluid has been linked to isolation of mycobacteria from human samples – but these are mostly false positive isolates collected through the contaminated scopes. Hot tub lung is a rarer condition that has been linked to exposure to contaminated water. However the vast majority of NTM infections have not necessarily been linked to environmental exposure, is less well defined and the subject of ongoing research. Genotyping studies matching environmental isolates and human isolates are needed and have not been conclusive to date.

“stipulates” is the wrong word here.

The last two paragraphs are also a problem. Have you or others shown that boiling of drinking water can reduce isolation of NTM? What is the evidence that drinking water is the most important route of human and animal exposure to NTM? Soil and water are so closely related that it may be gardening and use of wet soil in agriculture that is more important.

There are several minor typographical and grammatical errors that need attention, but given that the paper needs to be revised I have not commented on these at this stage.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.